Characterization of a new plasma membrane-associated 
esto-5'-phosphodiesterase/nucleotide-pyrophosphatase 
from rat hepatocarcinoma AS-30D cells

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We have identified in plasma membrane fractions isolated from rat hepatocarcinoma AS-30D ascites cells three glycoproteins of 125 kDa, 115 kDa and 105 kDa (gp125, gp115 and gp105) which become adenylylated using ATP as substrate, most readily in the presence of EDTA. The gp115 becomes also phosphorylated. The adenylylation of these tumor glycoproteins was much lower than that of a group of analogous adenylylatable glycoproteins (gp130, gp120-gp110 dimer and gp100) present in normal rat liver plasma membrane. The tumor glycoproteins were reversibly O-adenylylated at threonine residues, as was the case for their normal rat liver counterparts. The tumor gp115, and the gp120-gp110 dimer from normal rat liver were both isolated using either ATP-affinity chromatography and/or AMP-affinity chromatography. The gp120-gp110 dimer from normal rat liver was identified as the plasma cell differentiation antigen-1 (PC-1 protein), an ecto-5'- phosphodiesterase/nucleotide-pyrophosphatase (5'-PDE/NPPase). The gp115 from tumor cells also exhibited Zn2+-stimulated 5'-PDE and NPPase activities in alkaline conditions, although it appears to be distinct from the PC-1 protein. We have determined that the gp115 is an ecto-enzyme that catalyzes the hydrolysis of extracellular ATP, since its adenylylation and phosphorylation were detected in intact cells using extracellularly added [α-32P]ATP or [γ-32P]ATP, respectively, in the absence of any permeabilizing agent.

Key words: Adenylylation, AS-30D tumor cells, Ecto-5'-phosphodiesterase/nucleotide-pyrophosphatase, Hepatocarcinoma.

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In previous studies, we reported the presence of a set of glycoproteins (gp130, gp120-gp110 dimer, gp100 and gp86) in the plasma membrane of normal rat liver which became labeled in the presence of [α-32P]ATP (6, 28-30). We demonstrated that the gp86 was a phosphodiesterase forming an adenylylated catalytic intermediate, although it was uncertain at that time whether the labeled gp130, gp120-gp110 dimer and gp100 were also adenylylated intermediates of typical phosphodiesterases. This uncertainty was due to the low rate of turnover observed upon chase of the bound [32P]AMP by an excess of non-labeled ATP (28). On the other hand, in the cell plasma membrane of the rat hepatocarcinoma AS-30D we detected the presence of three adenylylatable proteins of slightly different molecular masses than their normal rat liver counterparts (6). However, the nature and physiological functions of these proteins remained elusive.

It is well known that 5′-phosphodiesterases form adenylylated catalytic intermediates (17, 27, 28). Moreover, a 130 kDa glycoprotein from bovine liver plasma membrane (21, 22) that becomes adenylylated using ATP, and that cross-reacts with antibodies against the plasma cell differentiation antigen-1 (PC-1 protein) (32, 33) has been described. The PC-1 protein is a 5′-phosphodiesterase/nucleotide-pyrophosphatase (5′-PDE/NPPase) that is structurally formed by two identical polypeptide chains bound by disulfide bridges, with molecular masses ranging from 115 kDa to 130 kDa depending on the species (2, 3, 9, 10, 25, 26, 36). Its coding sequence has been determined in mouse and human, and its protein product has a single transmembrane segment, a short intracellular N-terminal domain, and a large extracellular C-terminal domain containing the ATP catalytic site and one or two potential “E-F hand” Ca2+-binding sites (5, 9, 35, 36). Therefore, the PC-1 protein utilizes extracellular ATP as substrate (2). This enzyme belongs to a family of related mammalian ecto-enzymes whose encoding sequences have been reported (38, 39).

In this work, we have investigated whether the adenylylatable proteins detected in the plasma membrane from rat hepatocarcinoma AS-30D cells (6) are phosphodiesterases. Thus, we have demonstrated that the gp115 from these tumor cells is indeed a 5′-PDE/NPPase but it is distinct from the gp120-gp110 dimer (PC-1 protein) from normal rat liver plasma membrane. We also demonstrate that the tumor gp115 is an ecto-enzyme that catalyzes the hydrolysis of extracellular ATP.

Materials and Methods

Reagents.– [γ-32P]ATP and [α-32P]ATP were purchased from ICN, and thymidine 5′-monophosphate p-nitrophenyl ester (pNP-5′-T), ATP-agarose and AMP-agarose were obtained from Sigma. The polyclonal R244 antibody (36) against the mouse PC-1 protein, and the monoclonal 4H4 antibody (2) against the C-terminus of the human PC-1 protein were kindly provided by Dr. James W. Goding.

Preparation of plasma membrane fractions.– Plasma membrane fractions from normal rat liver and AS-30D tumor cells (31) were prepared as previously described up to the first discontinuous sucrose gradient centrifugation (29). The 5′-nucleotidase and the ouabain-sensitive Na+,K+-ATPase activities were assayed as described (12, 23).